

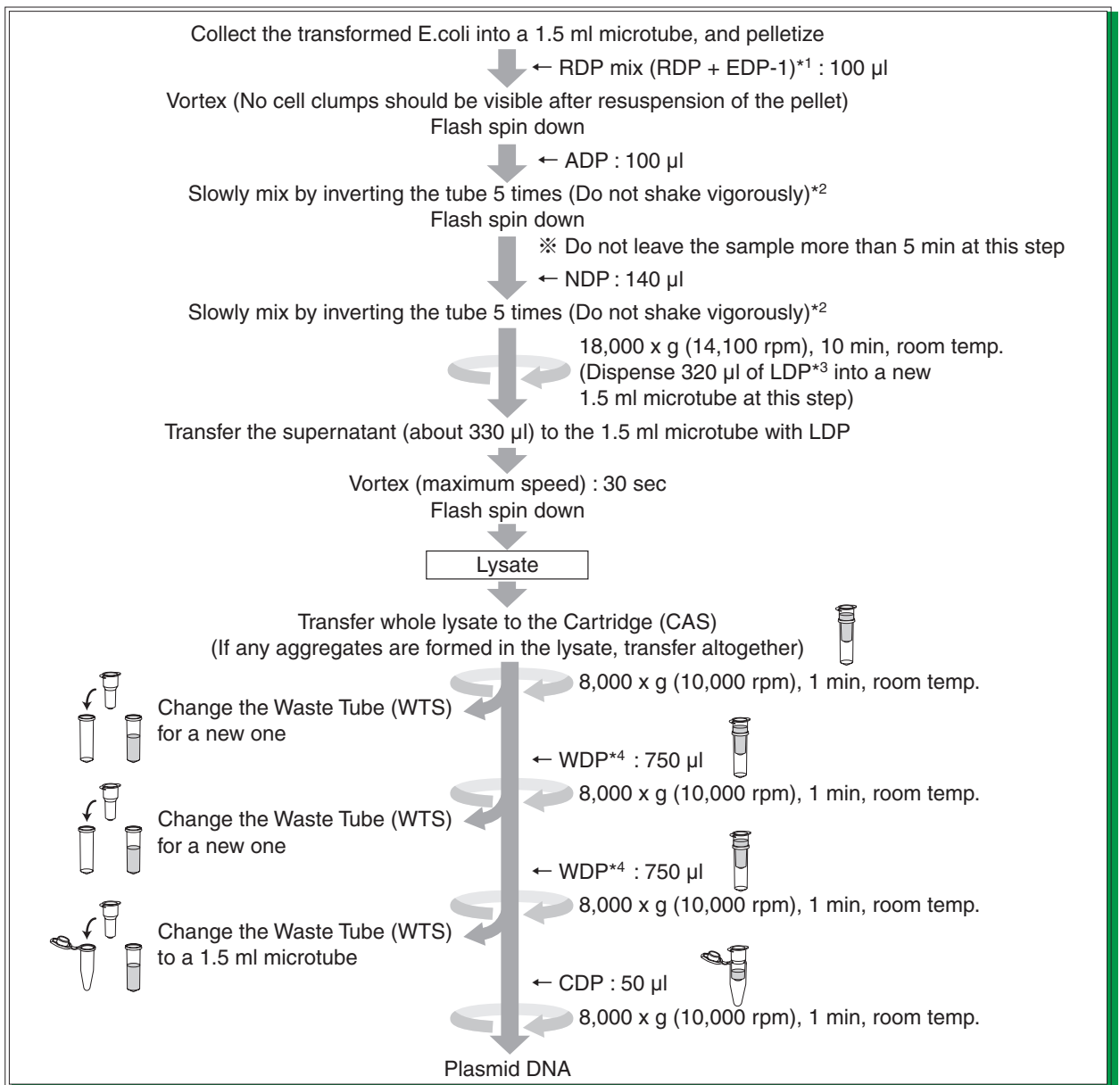


QuickGene Series **Application Guide**

**Plasmid DNA Extraction from E. coli**

Kit : QuickGene SP kit Plasmid II (Spin method)

**Protocol**



\*1 : Before starting an extraction experiment, add total amounts of EDP-01 to RDP bottle, and mix well. In the case of storing RDP mix, it is recommended to preserve it under refrigeration (2-8°C) and use within 6 months.

\*2 : After addition of ADP or NDP, immediately mix by inverting the tube 5 times.  
Vigorous mixing results in the copurification of much of genomic DNA. Too slow mixing causes inadequate blending of liquids, resulting in deterioration in the yield of plasmid DNA.

\*3 : Add 44 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

\*4 : Add 200 ml of >99% ethanol into the bottle and mix well by gently inverting the bottle before use.

\* Perform extraction within 30 min after lysate preparation.

## Results : Extraction of Plasmid DNA from transformed E. coli

Plasmid DNA was extracted from 1 ml over-night culture of transformed E. coli in LB medium using QuickGene SP kit Plasmid II .

E. coli : DH5  $\alpha$  ( $1 \times 10^9$ )

Vector : pBlueScript II

Insert : GAPDH about 1 Kb

### ● The yield and purity of plasmid DNA

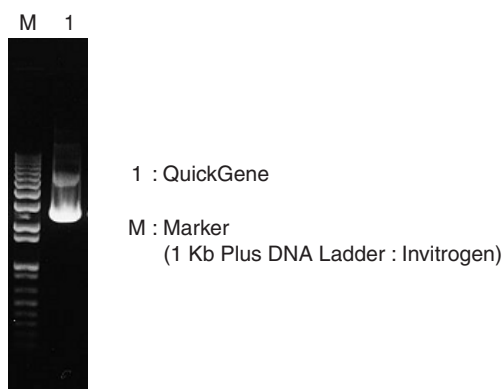
Kit	Yield	A <sub>260/280</sub>	A <sub>260/230</sub>
QuickGene	30.9 $\mu$ g	2.00	2.43

A<sub>260/280</sub> : The ratio indicates the purity of nucleic acid from protein contamination (A<sub>260/280</sub> >1.7).  
(Protein contamination decreases the ratio.)

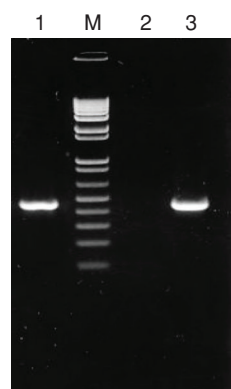
A<sub>260/230</sub> : The ratio indicates the purity of nucleic acid from chaotropic salt (guanidium salt) contamination.  
(Guanidium salt contamination decreases the ratio.)

The use of QuickGene SP kit Plasmid enables the high-yield and high-purity extraction of plasmid DNA from transformed E. coli.

### ● Electrophoresis of plasmid DNA



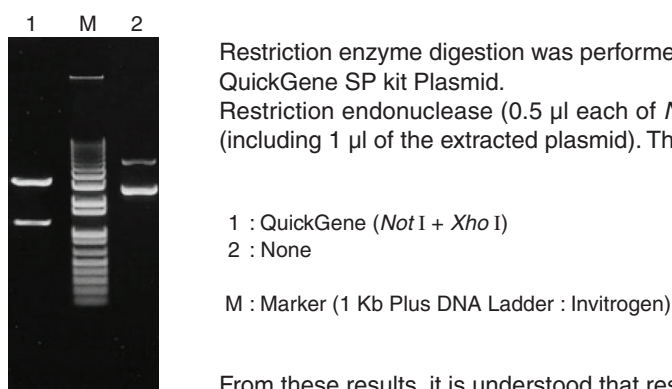
### ● PCR



PCR was performed on 5 ng of template extracted with QuickGene SP kit Plasmid using GAPDH as a target.

PCR amplification is possible from 5 ng of template.

### ● Restriction enzyme digestion with *Not* I and *Xho* I



Restriction enzyme digestion was performed for plasmid DNA extracted from transformed E. coli using QuickGene SP kit Plasmid.

Restriction endonuclease (0.5  $\mu$ l each of *Not* I and *Xho* I) were added to 10  $\mu$ l of a reaction solution (including 1  $\mu$ l of the extracted plasmid). Then it was incubated for 2 hours at 37°C.

From these results, it is understood that restriction endonuclease cleavage is practicable.

#### \* Trademark and exclusion item

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